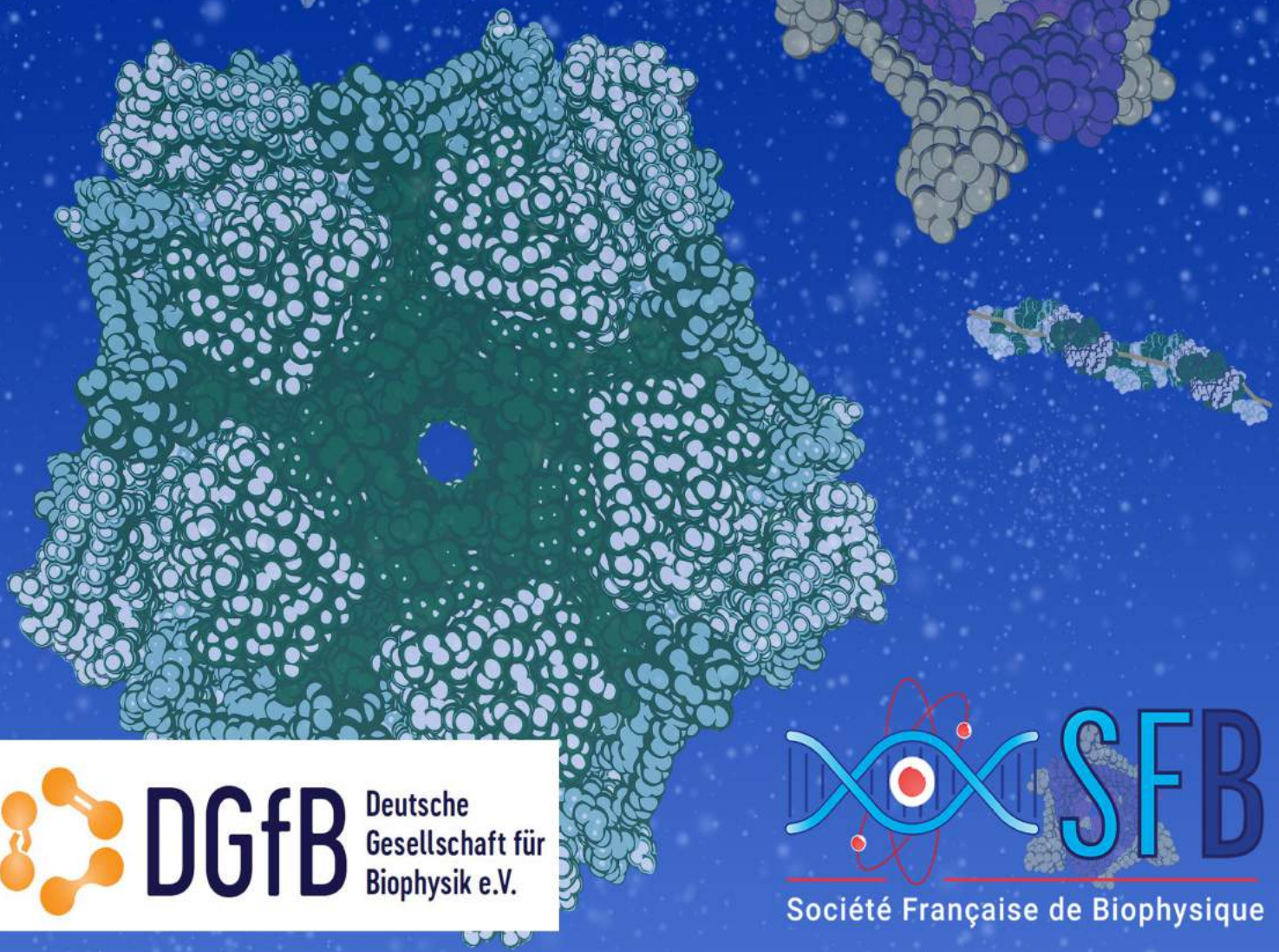
Joint Meeting of the French and German Biophysical Societies

Structure and Dynamics of Biomolecules

14 to 16 February 2019
Hünfeld, Germany



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We appreciate the support of the French Biophysical Society (SFB) and the German Biophysical Society (DGfB).

Structure and Dynamics of Biomolecules

Thursday 14.2.2019

12:30 – 15:00		*Registration*
15:00		*Welcome*
Session 1		**Cell walls and membrane remodeling**
15:05	Sven van Teefelen	Molecular and physical determinants of cell shape in bacteria
15:35	Jonas Ries	High-throughput superresolution microscopy of endocytosis – linking molecular architecture and mechanics of a protein machinery
16:05 - 16:30		*Coffee break*
Session 2		**Membrane reorganisation**
16:30	Kerstin Blank	Coiled Coils as Molecular Force Sensors
16:50	Tomas Fessl	A single-molecule study of the Sec machinery
17:10	Christian Sieben	Influenza A viruses use multivalent sialic acid clusters for cell binding and receptor activation
17:30	Christine Doucet	Nanoscale Organization of Tetraspanins during HIV-1 budding by correlative dSTORM/AFM
18:00 – 19:30		*Dinner*
Session 3		**Nucleus and Nucleoid Organisation**
19:30	Marcelo Nollmann	Visualizing DNA organization at the nanoscale
20:00	Joanna Timmins	Nucleoid organization and dynamics in *Deinococcus radiodurans*
20:30	Johannes Stigler	Protein-DNA interactions of SMC complexes observed with single molecule methods
20:50		*Get together*

Friday 15.2.2019

8:00 -9:00		*Breakfast*
Session 4		**Nucleic Acids I**
9:00	Terence Strick	Single-molecule approaches to the study of DNA repair: the case of NHEJ
9:30	Axel Innis	Sensing of the non-proteinogenic amino acid L-ornithine by nascent *Escherichia coli* SpeFL
10:00	Avin Ramaiya	Sponsor presentation: LUMICKS
10:10	Siva Subramanian	Insights into the mechanisms of action by Redβ during homologous recombination
10:30 – 11:00		*Coffee break*
Session 5		**Protein folding and IDPs**
11:00	Sigrid Milles	The Measles virus phosphoprotein – an intrinsically disordered chaperone that regulates nucleocapsid assembly
11:30	Simon Ebbinghaus	Quantification and modulation of in-cell protein folding stability
12:00	Ferdinando Ciceri	Sponsor presentation: Mad City labs
12:10	Ganesh Agam	Interplay of p53 conformation by Hsp40, Hsp70 and Hsp90 studied with spFRET
12:30 – 13:30		*Lunch break*
Session 6		**Enzyme mechanisms**
13:30	Guillaume Drin	An electrostatic switching mechanism controls the lipid exchange activity of an OSBP-related protein
14:00	Irina Gutsche	An enterobacterial stress response triad from a cryo-EM perspective
14:30	Petra Hellwig	Evidence for a distinct electron transfer mechanism in bd oxidase from *Geobacillus thermodenitrificans*
14:50	Helgo Schmidt	The CryoEM structure of the *Saccharomyces cerevisiae* ribosome maturation factor Rea1
15:10	Albert Weixlbaumer	Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation
15:30 – 16:30		*Coffee break*
Session 7		**Membrane proteins**
16:00	Thorben Cordes	Conformational plasticity in substrate-binding proteins underlies selective transport in ABC importers
16:30	Indra Schröder	Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral K+ channel
17:00	Olivier Fisette	The atomistic face of the human MHC-I peptide-loading complex
17:20	Poster flash talks	
18:00-19:00		*Dinner*
19:00-22:00	Poster session with drinks and snacks	

Saturday 16.2.2019

8:00-9:00		*Breakfast*
Session 8		**Nucleic acids II**
9:00	Sarah Willkomm	SLAM-FRET provides insights into the conformation landscape of human Argonaute 2
9:30	Mathias Pasche	Sponsor presentation: Photometrics
9:40	Dominique Burnouf	Biophysical approaches for the development of new antibiotics
10:00-10:20		*Coffee break*
Session 9		**Nucleic acids III**
10:20	David Dulin	High throughput magnetic tweezers to study RNA virus replication kinetics at the single molecule level
10:50	Ingrid Teßmer	Mechanistic insight on the alkyltransferase-like protein function in alkyl-DNA lesion repair from AFM imaging
11:20	Jan Lipfert	Multiplexed Magnetic Tweezers: From DNA Mechanics to Retroviral Integration
11:40	Johannes Hohlbein	Monitoring target search of a CRISPR-Cas system *in vivo*
12:00	Mikayel Aznauryan	Folding dynamics of G-quadruplex DNA in dilute and molecularly crowded environments
12:20 – 13:30		*Lunch break*
Session 10		**New technical developments**
13:30	Sonja Schmid	Single Protein Dynamics: From Fluorescence to Electrical Detection
14:00	Felix Rico	Protein mechanics probed by high-speed force spectroscopy
14:30	Ulrike Endesfelder	Exploring cell biology on a molecular level: Live-cell and quantitative localization microscopy
15:00	Arindam Ghosh	Graphene – MIET: Optically Measuring Distances with Ångström Resolution
15:20-15:40		*Coffee break*
Session 11		**Membrane complexes and Proteins**
15:40	Ingrid Chamma	Investigating synaptic adhesion dynamics and nano-organization using small monomeric binders
16:10	Marie-Lise Jobin	Spatial organisation and dynamics of GPCR signalling revealed by single-molecule fluorescence microscopy
16:40	Robert B. Quast	Structural dynamics of single metabotropic glutamate receptors
17:00	Francesco Pedaci	Mechano-sensitivity of the bacterial flagellar motor
17:20	Closing remarks and Poster Prizes	
18:00-19:00		*Dinner*

Sunday 17.2.2019

8:00-9:00	*Breakfast and Departure*

Abstracts

Talk abstracts are sorted chronologically according to the program.
Poster abstracts are sorted alphabetically.

Legend:

I = Invited talk
S = Selected talk
C = Sponsor presentation
P = Poster
T = Travel award winner

I 1	*Molecular and physical determinants of cell shape in bacteria*
	Sven van Teefelen

Bacterial cell shape and cell volume are physically determined by the peptidoglycan cell wall. For cell-envelope expansion during growth the cell wall must be enzymatically cleaved, and new material must be inserted. In many rod-shaped bacteria such as our model organism *Escherichia coli* cell-wall remodeling takes place at multiple sites along the side walls of the cell. Here, I will discern how envelope geometry, cell-wall architecture, and mechanical forces contribute to macroscopic cell shape by affecting the spatial distribution and activity of different cell-wall-modifying enzymes and the bacterial cytoskeleton.

I 2	*High-throughput superresolution microscopy of endocytosis – linking molecular architecture and mechanics of a protein machinery*
	Jonas Ries

Clathrin-mediated endocytosis is an essential cellular function of all eukaryotes. It relies on a self-assembled macromolecular machine of over 50 different proteins in tens to hundreds of copies that mediate vesicle formation. How so many proteins can be organized to produce endocytic vesicles with high precision and efficiency is not understood. To address this gap, we developed high-throughput superresolution microscopy to reconstruct the nanoscale structural organization of 23 endocytic proteins from over 100,000 endocytic sites in yeast. This allowed us to visualize where individual proteins are localized within the machinery throughout the endocytic process.

By combining superresolution imaging, live-cell microscopy and Brownian dynamics simulations, we aim to identify the architectural features that allow the endocytic machinery to create vesicles with high efficiency and robustness. We found that actin filament nucleation is pre-patterned by a nucleation nanotemplate, which directly links molecular organization to the mechanics of endocytosis, and might represent a general design principle for directional force generation in other membrane remodeling processes such as during cell migration and division.

Mund, M., van der Beek, J. A., Deschamps, J., Dmitrieff, S., Monster, J. L., Picco, A., et al. “Systematic analysis of the molecular architecture of endocytosis reveals a nanoscale actin nucleation template that drives efficient vesicle formation,” Cell (2018).

I 3	*Coiled Coils as Molecular Force Sensors*
	Kerstin Blank

Melis Goktas,[1] Alberto Sanz de León,[1] Patricia López García,[1] Isabell Tunn,[1] Emilia Grad,[1,2] Zeynep Atris,[1] Kerstin G. Blank[1]

[1] Max Planck Institute of Colloids and Interfaces, Mechano(bio)chemistry, Potsdam, Germany
[2] Radboud University, Molecular Materials, Nijmegen, The Netherlands

Coiled coils (CCs) are highly abundant motifs in structural proteins. Consisting of two (or more) α-helices wound around each other in a superhelical fashion, they represent essential structural elements of the cytoskeleton and the extracellular matrix (ECM). Considering their function as mechanical building blocks, surprisingly little is known about the structural determinants that define their molecular mechanical properties. Using atomic force microscope (AFM)-based single-molecule force spectroscopy, we have established the sequence-structure-MECHANICS relationship of a series of synthetic CCs. We show that CC mechanical stability depends on coiled coil length [1], helix propensity and hydrophobic core packing [2], as well as on the pulling geometry. Based on this knowledge, we have developed a library of CCs with tuneable mechanical properties. This library now serves as the basis for the development of CC-based molecular force sensors for 2D cell culture applications and allows for determining the threshold force for cell attachment. Towards 3D cell culture systems, we are developing poly(ethyleneglycol)-based hydrogels where the CCs act as mechanosensitive and self-reporting crosslinks [3]. Equipped with a fluorescence reporter system, these CC building blocks will allow for observing mechanical processes at the cell-material interface at the molecular level in real-time.

References

1. M. Goktas, C. Luo, R. M. A. Sullan, A. E. Bergues-Pupo, R. Lipowsky, A. Vila Verde, K. G. Blank (2018) Molecular Mechanics of Coiled Coils Loaded in the Shear Geometry. Chem. Sci. 9:4610
2. P. Lopez Garcia, M. Goktas, A. E. Bergues-Pupo, B. Koksch, D. Varon-Silva, K. G. Blank. Structural Determinants of Coiled Coil Mechanics, submitted
3. Tunn, A. S. de Léon, K. G. Blank, M. J. Harrington (2018) Tuning coiled coil stability with histidine-metal coordination. Nanoscale 10:22725

S 1	*A single-molecule study of the Sec machinery*
	Tomas Fessl

Tomas Fessl, Joel Crossley, Matthew Watson, Daniel Watkins, Peter Oatley, Robin Corey, William Allen, Steve Baldwin, Sheena E Radford, Ian Collinson, Roman Tuma

Most outer and inner membrane proteins in bacteria are transported and inserted by the ubiquitous Sec machinery (SecYEG complex in *E. coli*) which resides in the plasma membrane. Many proteins are transported post-translationally via the SecYEG channel at the expense of ATP hydrolysis by the peripheral membrane protein SecA and in concert with the trans-membrane proton motive force.

Using a combination of biochemical, computational and single molecule fluorescence techniques we have discovered a two-way communication between SecA ATPase and SecYEG channel, and proposed a Brownian motor model of translocation. We have extended this approach to investigate initiation of translocation and to estimate the translocation rate. FRET based recurrence analysis of freely diffusing single proteoliposomes improved the time resolution (0.1-50 ms time scale) and allowed to follow the initial unlocking and opening of the channel and the dynamics of the lateral gate.

S 2	*Influenza A viruses use multivalent sialic acid clusters for cell binding and receptor activation*
	Christian Sieben

Christian Sieben, Erdinc Sezgin, Christian Eggeling, Suliana Manley

Influenza A virus (IAV) binds its host cell using the major viral surface protein hemagglutinin recognizing sialic acid (SA), a plasma membrane glycan that functions as the specific primary attachment factor. Since SA alone cannot fulfil a signalling function, the virus needs to activate downstream factors in order to trigger endocytotic uptake. Recently, the epidermal growth factor receptor (EGFR) was shown to be activated by and transmit IAV entry signals. However, how IAV engages and activates EGFR remained largely unknown. We used quantitative super-resolution microscopy to study the lateral organization of both, IAV attachment factors as well as its functional receptor at the scale of the virus-cell interface (100 nm). We show that SA and EGFR are organized in partially overlapping submicrometer clusters in the apical plasma membrane of permissive A549 cells. Within SA domains, that are distinct of microvilli, the local SA concentration, a parameter that directly influences virus-cell binding, strongly increases towards the cluster center, thereby representing a multivalent virus-binding platform. Our quantitative cluster analysis allowed us to simulate virus membrane movement revealing that IAVs distinct membrane motility is dominated by the local SA concentration, which could be confirmed by live cell single- virus tracking. In contrast to SA, for EGFR we find clusters of rather low molecule abundance. Virus binding activates EGFR but interestingly this process occurs without a major lateral EGFR redistribution, suggesting the activation of preformed long-lived clusters. Taken together, our results provide a first step towards understanding the nanophysiology of influenza virus infection. We are able to relate the structural organization of the cell surface with its functional role during virus-cell binding and receptor activation.

S 3	*Nanoscale Organization of Tetraspanins during HIV-1 budding by correlative dSTORM/AFM*
	Christine Doucet

Selma Dahmane, Christine Doucet, Antoine Le Gall, Célia Chamontin, Patrice Dosset, Marylène Mougel, Marcelo Nollmann, Pierre-Emmanuel Milhiet

Membrane partition and remodeling play a key role in numerous cell mechanisms, especially in viral replication cycles where viruses subvert the plasma membrane to enter and escape from the host cell. Specifically assembly and release of HIV-1 particles require specific cellular components, which are recruited to the egress site by the viral protein Gag. We previously demonstrated that HIV-1 assembly alters both partitioning and dynamics of the tetraspanins CD9 and CD81, which are key players in many infectious processes, forming enriched areas where the virus buds. In this study we correlated super resolution microscopy mapping of tetraspanins with membrane topography delineated by atomic force microscopy (AFM) in Gag-expressing cells. We revealed that CD9 is specifically trapped within the nascent viral particles, especially at buds tips, and that Gag mediate CD9 and CD81 depletion from the plasma membrane. In addition, we showed that CD9 is organized as small membrane assemblies of few tens of nanometers that can coalesce upon Gag expression.

I 4	*Visualizing DNA organization at the nanoscale*
	Marcelo Nollmann

Eukaryotic chromosomes are organized in multiple scales, from nucleosomes to chromosome territories. Recently, genome-wide methods identified an intermediate level of chromosome organization, topologically associating domains (TADs), that play key roles in transcriptional regulation. However, these methods cannot directly examine the interplay between transcriptional activation and chromosome architecture while maintaining spatial information. Here, we present a multiplexed, sequential imaging approach (Hi-M) that permits the simultaneous detection of chromosome organization and transcription in single nuclei. This allowed us to unveil the changes in 3D chromatin organization occurring upon transcriptional activation and homologous chromosome un-pairing during the awakening of the zygotic genome in intact Drosophila embryos. Excitingly, the ability of Hi-M to explore the multi-scale chromosome architecture with spatial resolution at different stages of development or during the cell cycle will be key to understand the mechanisms and consequences of the 4D organization of the genome.

I 5	*Nucleoid organization and dynamics in Deinococcus radiodurans*
	Joanna Timmins

Kevin Floc'h, Françoise Lacroix, Pascale Servant, Jean-Philippe Kleman, Dominique Bourgeois and Joanna Timmins.

In all organisms, genomic DNA is compacted several orders of magnitude and yet must remain accessible for essential DNA-related processes including DNA replication, repair and transcription. Most of our knowledge of the spatial organization and dynamics of nucleoids originates from studies of rod- or crescent-shaped bacteria. We have used *Deinococcus radiodurans*, a relatively large, spherical bacterium, well-known for its exceptional radioresistance, to study the structure and dynamics of the nucleoid. Using advanced microscopy approaches, we have determined that *D. radiodurans* increases in size throughout its cell cycle through growth of both septal and peripheral cell walls, and have revealed that its nucleoid is highly compact at all times, but also surprisingly dynamic, adopting six distinct configurations, including the previously described toroid, as it progresses through its cell cycle. This remarkable plasticity is permitted by the loose binding of the highly abundant histone-like HU protein to the genomic DNA, as revealed by single-molecule and ensemble measurements of HU dynamics. Moreover, by mapping specific chromosome loci, we show that the largest chromosome of *D. radiodurans* presents a novel arrangement in which *oriC* loci are radially distributed around *ter* sites that are largely clustered at the centre of cells for most of the cell cycle. Taken together, these results demonstrate that nucleoids are highly organized and dynamic structures, which are tightly regulated by cell shape and cell cycle progression.

S 4	*Protein-DNA interactions of SMC complexes observed with single molecule methods*
	Johannes Stigler

Sister chromatid cohesion, chromosome segregation and the regulation of gene expression are directly influenced by interactions between SMC complexes and DNA. However, the detailed molecular mechanism of how SMC complexes interact with DNA are still controversial. We demonstrate in single molecule experiments that the eukaryotic SMC complex cohesin forms topological contacts with DNA. Our observations let us estimate the size of the lumen that is encompasses DNA. We further characterize the effects of nucleotides and SMC loading factors.

I 6	*Single-molecule approaches to the study of DNA repair: the case of NHEJ*
	Terence Strick

Single-molecule experiments fall into two main categories: single-molecule nanomanipulation (which allows one to detect the activity of proteins working on substrates) and single-molecule fluorescence (which allows one to detect the presence of proteins on substrates). Despite their name, single-molecule approaches provide the means to reconstruct complex, multicomponent, multistep reactions in real-time. This allows one to reach deep inside reaction pathways to query the detailed kinetics of individual steps, without a need for artificial synchronization of a reaction. These ideas are applied to the compelx process whereby the cell repairs DNA double-strand breaks (DSBs) by non-homologous end-joining (NHEJ). We will conclude by discussing the ways in which single-molecule nanomanipulation and single-molecule fluorescence can contribute, either separately or combined into correlative assays, to the study of the assembly, activity, and disassembly of multicomponent systems in real-time.

I 7	*Sensing of the non proteinogenic amino acid L-ornithine by nascent Escherichia coli SpeFL*
	Axel Innis

Bacteria occupying the same niche compete for limited resources by adjusting the expression of their biosynthetic enzymes in response to fluctuating metabolite levels. In some cases, metabolites can be sensed directly by a ribosome engaged in the synthesis of a specialized polypeptide, known as an arrest peptide. These regulatory peptides remain trapped within the ribosome in their nascent state, leading to the formation of a stalled ribosome nascent chain complex on the mRNA. Translational arrest depends both on the amino acid sequence of the arrest peptide and, in some cases, on inducing levels of a particular metabolite. Although arrest peptides have been known for over three decades, the extent to which they are present in nature and the mechanisms by which they sense fluctuating metabolite concentrations are far from being understood. Here, I will present our efforts to understand the diversity of mechanisms employed by arrest peptides to detect changing concentrations of metabolite in their environment. Specifically, I will describe our recent structural and biochemical characterization of SpeFL, an arrest peptide that functions as an L-ornithine sensor in *Escherichia coli*.

C 1	*Sponsor presentation: LUMICKS* *Real-time single-molecule investigation of dynamic biological processes: From structure to function*
	Avin Ramaiya

Avin Ramaiya, Ann Mukhortava, Aida Llauro Portell, Roeland van Wijk, Andrea Candelli, Gerrit Sitters

Biological processes performed by proteins interacting with dynamic cytoskeletal structures and processing DNA/RNA are key to cell metabolism and life. Detailed insights into these processes allow understanding the molecular basis of life and the pathological conditions that develop when such processes go awry. Force spectroscopy on a single-molecule level permits exploring and manipulating these complex interactions to help understand their nature better.
The C-Trap™ is the world's first instrument that integrates optical tweezers, confocal/STED microscopy/ Interference Reflection Microscopy (IRM), and an advanced microfluidics in a truly correlated manner. The C-Trap™ enables live, simultaneous and correlative visualization and manipulation of molecular interactions with sub-picoNewton (pN) force resolution and microsecond temporal resolution.
Here, we present our latest applications of these technologies in studying protein (un)folding and conformational changes; DNA-protein interactions and genome modification; effects of mechanical stress on DNA/RNA structure; motility of cytoskeletal molecular motors; protein droplet and aggregation dynamics; and cell-receptor interactions. These can also easily be measured under different conditions e.g. temperatures, buffers and in the presence of small molecules.
These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that opens up new venues in many research areas

S 5	*Biochemical and biophysical insights into the mechanisms of action by Redβ during homologous recombination*
	Sivaraman Subramaniam

Sivaraman Subramaniam, Marcel Ander, Erik Schaeffer, Francis A. Stewart

Repair of DNA breaks by single-strand annealing (SSA) is a major mechanism for the maintenance of genomic integrity. SSA is promoted by proteins (single-strand-annealing proteins [SSAPs]) such as eukaryotic RAD52 and λ phage Redβ. These proteins use a short single-stranded region to find sequence identity and initiate homologous recombination. Using protein biochemistry and recombination assays, we have shown that C-terminally truncated Red β, whilst still able to promote annealing and nucleoprotein filament formation, is unable to mediate homologous recombination. As evaluated by co-immunoprecipitation experiments, the dsDNA recombination function relates to the Redα-Redβ interaction, which requires not only contacts in the C-terminal domain but also at the N-terminus. Mutations of critical amino acids affected either dsDNA recombination or both ssDNA and dsDNA recombination, indicating two separable functions: one critical for dsDNA recombination and the other for recombination per se. Using biophysical single molecule techniques, we have shown that homology is recognized by Redβ monomers that weakly hold single DNA strands together. Upon annealing, homodimerization of Redβ clamps the double-stranded region and nucleates nucleoprotein filament growth. In this manner, DNA clamping ensures and secures a successful detection for DNA sequence homology. Redβ clamp is characterized by a structural change and a remarkable stability against force up to 200 pN. Our findings not only present a detailed explanation for SSAP action but also identify the DNA clamp as a very stable, non-covalent, DNA-protein interaction. These data further advance Red recombination model and show that Redβ and RAD52 SSAPs share ancestral and mechanistic roots.

I 8	*The Measles virus phosphoprotein – an intrinsically disordered chaperone that regulates nucleocapsid assembly*
	Sigrid Milles

Sigrid Milles [(1)], Malene R Jensen [(1)], Carine Lazert [(2)], Serafima Guseva [(1)], Stefaniia Ivashchenko [(1)], Guillaume Communie [(1)], Damien Maurin [(1)], Guy Schoehn [(1)], Denis Gerlier [(2)], Rob WH Ruigrok [(1)], Martin Blackledge [(1)]

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Measles virus genome encapsidation is essential for viral replication and is controlled by the intrinsically disordered phosphoprotein (P) maintaining the nucleoprotein in a monomeric form (N) before nucleocapsid assembly. All paramyxoviruses harbor highly disordered amino-terminal domains (PNTD) that are hundreds of amino acids in length and whose function remains unknown. We previously demonstrated how the presence of a short 50 residue peptide of PNTD can prevent premature assembly of N during expression by forming a monomeric N0P construct that self-assembles into NC-like particles upon addition of RNA in vitro [1]. We now describe the structure and dynamics of the 90-kDa N0P complex comprising the full N and PNTD with a total of 450 disordered amino acids, at atomic resolution [2]. NMR relaxation dispersion reveals the existence of an ultraweak N-interaction motif, hidden within the highly disordered PNTD, that allows PNTD to rapidly associate and dissociate from a specific site on N while tightly bound at the amino terminus, thereby hindering access to the surface of N. Mutation of this linear motif quenches the long-range dynamic coupling between the two interaction sites and completely abolishes viral transcription/replication in cell-based minigenome assays comprising integral viral replication machinery. This description transforms our understanding of intrinsic conformational disorder in paramyxoviral replication. The essential mechanism appears to be conserved across Paramyxoviridae, opening unique new perspectives for drug development against this family of pathogens.

[1] Self-Assembly of Measles Virus Nucleocapsid-like Particles: Kinetics and RNA Sequence Dependence. Milles S, Jensen MR, Communie G, Maurin D, Schoehn G, Ruigrok RW, Blackledge M., Angew Chem Int Ed Engl. 2016

[2] An ultraweak interaction in the intrinsically disordered replication machinery is essential for measles virus function. Milles S, Jensen MR, Lazert C, Guseva S, Ivashchenko S, Communie G, Maurin D, Gerlier D, Ruigrok RWH, Blackledge M., Sci Adv. 2018

I 9	*Quantification and modulation of in-cell protein folding stability*
	Simon Ebbinghaus

Proteins fold and function in the densely crowded and highly heterogeneous cell, which is filled up to a volume of 40% with macromolecules. That under such conditions cells can keep their proteome folded and organized without uncontrollable aggregation is a remarkable aspect of biology. In this talk, I will first discuss how the different cosolutes in the cellular milieu such as ions, crowders and osmolytes govern the protein folding equilibrium. I will thereby present a novel classification scheme of cosolute effects based on their thermodynamic fingerprints. This model is of fundamental importance to understand how the proteome stability is modulated by cellular processes, e.g. to understand how osmolytes or chaperones protect the proteome or how most destabilized proteins aggregate under different cell stresses. I will further present spectroscopic probes that explore the different cosolute effects directly in cells and show that cell stress can significantly modulate the folding equilibrium. Remarkably, protective cellular mechanisms such as the heat shock response or the regulatory volume increase are highly adapted to minimize the impact on the proteome. I will further present novel inhibitors for protein aggregation as a new therapeutic approach for neurodegenerative diseases.

C 2	*Sponsor presentation: MadCityLabs* *Modular Microscopy Systems for Single Molecule Imaging*
	Ferdinando Cicero

Single molecule approaches to understanding biological processes present many unique challenges for microscopy systems that conventional microscope platforms were not designed to meet. These challenges can be grouped into 3 broad categories: 1) a greater need for open and flexible access to optical pathways; 2) more stringent requirements for precise control over sample position; and 3) the paramount importance for stability in the overall system. To serve the important emerging needs of single molecule approaches, we have developed an open, modular, flexible, and extensible microscopy platform, which we call the RM21™. It is specifically designed to meet these challenges, while also making customization and innovation straightforward. Very importantly, we have leveraged our expertise in precise positioning in all aspects of this platform: the RM21™ is designed from the bottom up, with system stability firmly in mind, as well as full integration with an array of options for both micro- and nanopositioning stages. These design considerations enable precise and comprehensive control over sample positioning while maintaining overall system stability. We have also placed anchor points for cage-system mounting of widely available optomechanical components at convenient positions throughout this platform, supporting flexibility and ease of design, assembly, and alignment of desired final systems. Finally, we have developed and integrated both focal and 3-dimensional active drift-compensation systems to address the stability needs of single molecule experiments. We demonstrate how this platform can be configured to support several specific single molecule methods, and provide examples of how it can be extended to support others. We also use single molecule imaging to examine the sample positioning capabilities of these systems, as well as their stability with and without active drift compensation.

S 6	*Interplay of p53 conformation by Hsp40, Hsp70 and Hsp90 studied with spFRET*
	Ganesh Agam

Ganesh Agam, Vinay Dahiya, Johannes Buchner, Don C. Lamb

Tumor suppressor protein p53, is implicated in various cancer types. In cells, it is often found to interact with Hsp70 and Hsp90 chaperones. Various reports suggest the active role of these chaperones in modulating *in vivo* p53 activity . Despite the importance of these interactions, there is still lack of studies on how these chaperone systems work together to keep p53 *in vivo* functional. To understand the effect of these interactions on p53 conformation *in vitro* with single-molecule spFRET, we have designed a p53 FRET sensor . We found that the p53 alone is dynamic and unstable at physiological temperatures. Due to its intrinsic instability, it is more prone to interaction with Hsp40-Hsp70 chaperone system in presence of ATP at elevated temperatures. This leads to increase in unfolding of p53 by Hsp40-Hsp70 chaperones as temperatures increases to physiological temperatures. Hsp40 and Hsp70 bound unfolded p53 conformation changes towards more compact when allowed to interact with Hsp90. This hints the active role of Hsp90 in modulating the p53 conformation. The interplay seen in *in vitro* experiments, shed the light on how both the chaperone systems regulate the p53 conformation and susequently function in cells

I 10	*An electrostatic switching mechanism controls the lipid exchange activity of an OSBP-related protein*
	Guillaume Drin

Nicolas-Fréderic Lipp,[1] Romain Gautier[1], Maud Magdeleine[1], Maxime Renard[1], *Véronique Albanese[2],* Alenka Čopič [2] and Guillaume Drin[1]

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In eukaryotic cells, phosphatidylserine (PS) is made in the endoplasmic reticulum (ER), yet it is enriched in the plasma membrane (PM), where it provides negative charge and is critical for recruiting signaling proteins. In yeast, Osh6p and Osh7p, two lipid transfer proteins (LTPs), contribute to this accumulation by transporting PS from the ER to the PM via PS/phosphatidylinositol 4-phosphate (PI4P) exchange cycles between these compartments. A wide yet poorly documented assumption is that LTPs associate only transiently to organelle membranes to transport lipids efficiently. Regarding Osh6p/Osh7p, it is quite unknown how, at each cycle, they escape from the electrostatic attraction of the PM to move back to the ER. We addressed this issue by in vitro fluorescence-based assays and molecular dynamic simulations, backed on cellular observations. We show that Osh6p dissociates from an anionic membrane, like the PM, once it captures either PS or PI4P. The N-terminal lid of Osh6p, when closing the lipid-binding pocket upon lipid extraction, changes the electrostatic surface of the protein, thereby reducing its avidity for anionic membranes. Owing to this electrostatic switching mechanism, Osh6p keeps a fast exchange activity between weakly and highly negatively-charged membranes, i.e in a model system mimicking the ER/PM interface. Attenuation of the electronegativity of the lid weakens such a capacity. This study demonstrates how a LTP can self-limit its residency time on membranes in order to be competent.

I 11	*An enterobacterial stress response triad from a cryo-EM perspective*
	Irina Gutsche

This talk will summarize 15 years of our work on structure-function relationships of a protein triad – LdcI, RavA and ViaA - involved in enterobacterial stress responses. Acid stress-inducible lysine decarboxylase LdcI is a very well-known enzyme, scrutinized since 75 years because of the link between pathogenicity of enterobacteria and their ability to withstand aggressive pH environments in their human host. This acid stress response enzyme interacts with a AAA+ ATPase RavA which enables it to counteract acid stress even under starvation conditions. E. coli RavA and its operon partner, the VWA domain-containing protein ViaA, were recently proposed to play a role in the maturation of both respiratory Complex I and fumarate reductase. But what are the structures of LdcI, RavA and ViaA, how do these proteins interact with each other and with their cellular partners, what are their exact functions and how do they perform them, what are the molecular mechanisms of their function in antibiotic resistance and can the acquired knowledge be beneficial for drug design? To address these questions, we are using an integrated approach around cryo-EM but ranging from phenotypic analysis and molecular biology, through biochemistry, biophysics, X-ray crystallography and SAXS to optical imaging, cellular electron microscopy and cryo-electron tomography.

S 7	*Evidence for a distinct electron transfer mechanism in bd oxidase from Geobacillus thermodenitrificans*
	Petra Hellwig

A. Nikolaev, S. Safarian, A. Thesseling, H. Michel, T. Friedrich, F. Melin, P. Hellwig*

Cytochrome bd oxidases are solely present in the respiratory chain of bacteria, including several pathogens such as *Escherichia coli, Mycobacterium tuberculosis* or *Klebsiella pneumonia*. These oxidases are not only part of the respiratory chain within these bacteria and catalyze the reduction of oxygen to water, they are also believed to play a crucial role in the protection against oxidative stress, in their virulence, adaptability and antibiotics resistance. Depending on the type of quinone-binding site (so-called Q-loop) two subfamilies are currently distinguished: short and long Q-loop *bd*-oxidases.

In this study evidence is given for a distinct electron transfer mechanism between the *bd* oxidase from *E. coli* (long Q-loop) and the *bd* oxidase from *G. thermodenitrificans* (short Q-loop). Significant differences of the redox potentials of the hemes can be reported. Importantly, protein film voltammetry of *bd* oxidases, revealed an unusual electro catalytic behavior, pointing towards a pH dependent activation mechanism in the *G. thermodenitrificans* enzyme, that is absent *in E. coli*. Electrochemically induced vibrational spectroscopies confirm major differences in the active site of the two oxidases. The existence of different *bd* oxidase families is discussed.

S 8	*The CryoEM structure of the Saccharomyces cerevisiae ribosome maturation factor Rea1*
	Helgo Schmidt

Sosnowski P., Urnavicius L., Boland A., Fagiewicz R., Busselez J., Papai G. and Schmidt H.

The biogenesis of 60S ribosomal subunits is initiated in the nucleus where rRNAs and proteins form pre-60S particles. These pre-60S particles mature by transiently interacting with various assembly factors. The ~5000 amino-acid AAA+ ATPase Rea1 (or Midasin) generates force to mechanically remove assembly factors from pre-60S particles, which promotes their export to the cytosol. Here we present three Rea1 cryoEM structures. We visualise the Rea1 engine, a hexameric ring of AAA+ domains, and identify an α-helical bundle of AAA2 as a major ATPase activity regulator. The α-helical bundle interferes with nucleotide-induced conformational changes that create a docking site for the substrate binding MIDAS domain on the AAA +ring. Furthermore, we reveal the architecture of the Rea1 linker, which is involved in force generation and extends from the AAA+ ring. The data presented here provide insights into the mechanism of one of the most complex ribosome maturation factors

S 9	*Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation*
	Albert Weixlbaumer

Mo'men Abdelkareem, Charlotte Saint-André, Maria Takacs, Gabor Papai, Corinne Crucifix, Xieyang Guo, Julio Ortiz, Albert Weixlbaumer

Regulatory sequences or erroneous incorporations during DNA transcription cause RNA polymerase backtracking, and inactivation in all kingdoms of life. Reactivation requires RNA transcript cleavage. Essential transcription factors (GreA/GreB, or TFIIS) accelerate this reaction. We report four cryo-EM reconstructions of *Escherichia coli* RNA polymerase representing the entire reaction pathway: A backtracked complex (i); a backtracked complex with GreB before (ii), and after (iii) RNA cleavage; and a reactivated, substrate bound complex with GreB before RNA extension (iv).

Compared with eukaryotes, the backtracked RNA adopts a different conformation. RNA polymerase conformational changes cause distinct GreB states: i) a fully engaged GreB before cleavage; ii) a disengaged GreB after cleavage; and iii) a dislodged, loosely bound GreB removed from the active site to allow RNA extension. These reconstructions give insights on the catalytic mechanism and dynamics of RNA cleavage and extension, and suggest how GreB targets backtracked complexes without interfering with canonical transcription.

I 12	*Conformational and dynamical plasticity in substrate-binding proteins underlies selective transport in ABC importers*
	Torben Cordes

Substrate-binding proteins (SBPs) are associated with ATP-binding cassette (ABC) importers and switch from an open to a closed conformation upon substrate binding, thereby providing specificity to transport. We investigated the effect of substrates on the conformational dynamics of six different SBPs and the resultant impact on transport. Using single-molecule FRET, we reveal a hitherto unrecognized diversity of structural and dynamic plasticity in SBPs. We show that a unique closed SBP conformation does not exist for transported substrates. Instead, SBPs sample a range of conformations that activate transport. Certain ligands that are not transported leave the structure largely unaltered or trigger a conformation that is distinct from that of transported substrates, thus explaining the transport phenotype. Intriguingly, in some cases similar SBP conformations are formed by both transported and non-transported ligands. In this case, the inability to be transported arises from the slow opening of the SBP or the selectivity provided by the translocator domain. Our results reveal the complex interplay between ligand-SBP interactions, SBP conformational dynamics and substrate transport.

I 13	*Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral K^+ channel*
	Indra Schroeder

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Many K^+ channels exhibit selectivity filter gating, often voltage-dependent and independent from dedicated voltage-sensor domains. A plethora of experimental and theoretical literature has shown that this is likely modulated by the occupation of K^+ binding sites within the selectivity filter. We approached this topic by analyzing single-channel gating kinetics and by revealing details on ion occupation from the same set of lipid bilayer experiments.

Kcv channels are viral pore-only K^+ channels; their selectivity filter gating, as observed from *in vitro* expressed proteins in planar lipid bilayers, is modulated by both voltage and K^+. This sub-ms gating is beyond the canonical resolution of the experiments and was thus analyzed by fitting extended beta distributions to amplitude histograms (1). A global fit of the rate constants and the single-channel IV curves based on available atomistic models of ion transport was performed for different voltages and K^+ concentrations. The rate constant of channel closing correlates with the probability of three ions being in the filter instead of two (2).

The experimental results further point to a crucial role of flexibility in modulating selectivity filter closure rather than a direct conformational change. The role of the inherent flexibility for gating in Kcv channels is currently further explored by anisotropic network modelling.

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(2) Rauh, O., U. P. Hansen, D. D. Scheub, G. Thiel and I. Schroeder*. Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral K^+ channel. *Sci. Rep.*, 10406, 2018

S 10	*The atomistic face of the human MHC-I peptide-loading complex*
	Olivier Fisette

Olivier Fisette, Lars V. Schäfer

Antigens exposed at the cell surface by major histocompatibility complex class I (MHC-I) proteins enable self/non-self recognition by cytotoxic T cells, protecting the organism against viral infections and cancer-causing mutations. To perform their role, MHC-I must first be loaded with an antigenic peptide in the peptide-loading complex (PLC), a large multi-protein assembly whose atomic-level structure and dynamics are still poorly understood.

Using all-atom molecular dynamics (MD) simulations, we studied key elements of the human PLC, how they stabilise MHC-I and catalyse antigen selection, and how they assemble to form the PLC. By combining microsecond-timescale MD simulations with a recent 6-Å-resolution cryo-EM structure of the PLC, we obtained an atomistic model of the complete complex, in explicit solvent and in a membrane environment (1.5 million atoms). This model offers unprecedented insights into the structure and dynamics of the human antigen-loading machinery.

Our simulations explain how tapasin, a central component of the PLC, acts as both an MHC-I chaperone and a catalyst that accelerates the off-rate of low-affinity peptides to facilitate antigen triage (peptide editing). We also show how tapasin recruits the transporter associated with antigen processing (TAP) into the PLC via transmembrane interactions. Finally, truncating antigens or removing them from the MHC-I binding groove gives a spatially resolved map of MHC-I plasticity which reveals how peptide loading status affects key structural regions.

Taken together, our MD simulations explain experimental kinetics and mutagenesis data, and represent the first in-depth, atomic-level study of the mechanisms underlying the biological function of the PLC, an important step towards a better understanding of adaptive immunity.

I 14	*SLAM-FRET provides insights into the conformational landscape of human Argonaute 2*
	Sarah Willkomm

Sarah Willkomm, Leonhard Jakob, Alexander Gust, and Dina Grohmann

Human Argonaute 2 (hAgo2) is the key player of RNA interference (RNAi), a posttranscriptional mechanism that regulates a major portion of human genes, including a high number of genes involved in disease-related processes. To exert its function, hAgo2 binds short RNAs that guide the enzyme to its cognate mRNA targets via base complementarity. Incomplete complementarity between miRNA guides and target mRNA leads to the recruitment of additional proteins that promote mRNA decay or translational inhibition of the bound mRNA. Alternatively, direct cleavage of the bound mRNA by hAgo2 is possible. As common to transient protein-nucleic acids complexes, interactions have to be established and also disrupted to allow substrate and interaction partner exchange during the Ago activity cycle. This demands a certain degree of structural flexibility in hAgo2. X-ray crystal structures provided valuable information about the stable conformations that hAgo2 adopts upon binding of guide RNA and a short target RNA fragment. However, the dynamic aspect of hAgo2 action and the conformation hAgo2 adopts to bind longer target RNAs still remain to be elucidated. In order to conduct single molecule FRET measurements that shed light on the mechanistic details of the activity cycle of hAgo2 we developed the SLAM-FRET (**S**ite-specific **la**belling of endogenous **m**ammalian proteins for single-molecule FRET measurements) workflow *(1)*. This includes native state hAgo2 produced in human cells with all its necessary post-translational modifications like e.g. phosphorylation. Making use of this method, we were able to conduct smFRET measurements with site-specifically fluorescently labeled hAgo2 and/or labeled guide and target RNAs. This way, we observed the conformational evolution of hAgo2 throughout its activity cycle. Among others, we found that hAgo2 samples conformations until its nucleic acid binding partners are bound. However, also the ternary complex reveals a high degree of flexibility. Moreover, interaction partners like Dicer and TNRC6 appear to modulate the conformation of hAgo2. Hence, our data complement the structural information on hAgo2 and provide insights into the mode of interaction between hAgo2 and additional proteins that are part of the RNAi pathway.

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C 3	*Sponsor presentation: Photometrics* *The development of scientific CMOS cameras for low light, high speed and large field of view imaging*
	Mathias Pasche

Photometrics is the premier designer and manufacturer of high-performance CCD, EMCCD and sCMOS cameras as well as multi-channel imaging solutions for life science research. We are leading the industry for several decades with state-of-the-art imaging instrumentation. Tens of thousands of researchers around the globe rely on these cutting-edge imaging solutions to meet their most demanding quantitative research requirements.

Back-illuminated Scientific CMOS sensors are the future of camera technology which is already available today from Photometrics. Applying back-thinning to scientific-grade CMOS-sensors allows the production of cameras with a quantum efficiency as high as 95%.

The parallelisation of the readout electronics in the CMOS architecture enables manufacturers to build sensors which are up to 9x larger than conventional CCD- and EMCCD-based sensor. However even at this size, full chip framerates can be achieved which are well beyond CCD and EMCCD sensors.

Our two flagship products Prime 95B and Prime BSI showcase those latest developments having 95% quantum efficiency, framerates up to 82 fps at sensor diameters which can reach up to 25mm. This is all designed to achieve very low red noise levels of below 1.5 e-. In case of the Prime BSI we´ve recently implemented a read-out mode which utilizes correlated multi-sampling achieving a read noise of <1e- at a blazing 43fps.

The bias-level and signal quality across the entire field-of-view is well ahead of the competition.

All-together, the Prime series cameras will help to advance science enabling recordings of better quality data which produce more data at once due to the large field-of-view, streamlining experiments. The obtained data will be of higher and more reliable quality than what you´re used to.

S 11	*Biophysical approaches for the development of new antibiotics*
	Dominique Burnouf

C. André; A.M. Lobstein; I. Martiel; F. Veillard; P. Wolff; G. Guichard; V. Oliéric; J.M. Reichhart; J. Wagner and D. Burnouf

Bacterial resistance to antibiotics has become a major concern for human health. One approach to tackle this global public health problem is to identify new bacterial molecular targets and to develop molecules that will block their physiological functions and ultimately trigger bacterial cell death.

We have identified such a new target and have developed peptides that specifically interact with it.

This presentation will focus on the biophysical (ITC and X-Rays crystallography) and biochemical evaluation of the in vitro activity of these peptides. Moreover, their antibacterial activity is further demonstrated in an vivo infectious model.

I 15	*High throughput magnetic tweezers to study RNA virus replication kinetics at the single molecule level*
	David Dulin

The use of synthetic, primed templates to monitor the kinetics of nucleotide addition in ensemble experiments has been invaluable to the development of our current understanding of RdRp mechanism. However, the context in which an elongating PV RdRp functions in the cell is not tens of nucleotides but thousands of nucleotides. Ensemble experiments do not offer the ability to monitor even 10 cycles of nucleotide addition, with often only a subset of the four NTPs, in a manner that would permit interrogation of the elementary steps of the nucleotide-addition cycle in a meaningful way. We have developed a high-throughput single-molecule magnetic tweezers approach to study nucleotide addition by RdRps (Dulin et al., Cell Reports 2015, 2017). Using this approach, data for as many as 1000 individual RdRp molecules elongating over a distance of 2800 nt can be acquired with near single-nucleotide resolution.

Using this single-molecule technique, we show that the PV RdRp is able to incorporate more than 2000 nt efficiently and without dissociating. Pauses of variable duration are readily observed. Use of dwell-time analysis to compare different experimental conditions (changes in nucleotide concentration, use of nucleotide analogues, or RdRp derivatives) revealed the existence of a discrete, error-prone kinetic pathway for RV RdRp, similar to that observed for Φ6 RdRp (Dulin et al., Cell Reports 2015). Temperature dependent study of viral replication indicates that the temperature is an important factor for fast replication with poliovirus RdRp, but not with Φ6 RdRp (Seifert et al., in preparation). Analysis of various antiviral nucleotides, e.g ribavirin, revealed unexpected differences in mechanisms of binding/incorporation and post-incorporation pausing. We are confident that this approach will add substantively to our understanding of polymerase mechanism and will become the new state of the art for polymerase enzymology.

I 7	*Mechanistic insight on the alkyltransferase-like protein function in alkyl-DNA lesion repair from AFM imaging*
	Ingrid Tessmer

DNA repair is vitally important for the maintenance of genomic stability and thus the prevention of cancer, premature ageing, or cell death. Alkylation of guanine bases, in particular, is one of the most highly mutagenic and cytotoxic DNA lesions and is repaired by the O6-guanine DNA alkyltransferase, AGT (also known as MGMT, methyl-guanine methyltransferase). In addition, many organisms possess an alkyltransferase-like protein (ATL), which cannot remove alkyl-groups from damaged bases. Instead, ATLs stably bind to alkyl-guanine with high affinity. There has been strong evidence that the thus marked lesion may then be recognized and repaired by the nucleotide excision repair (NER) system. The high structural similarity of ATLs with the catalytic, DNA binding domain of AGT, and the bridging of different DNA repair mechanisms make ATL an extremely interesting system to study in terms of DNA lesion search and recognition approaches. We investigated ATL-DNA interactions and NER recruitment by ATL using a range of biophysical and biochemical methods. In particular, the direct, molecular resolution and distinction of different types of DNA bound complexes using single molecule imaging by atomic force microscopy (AFM) allows us to draw important conclusions on ATL function.

S 12	*Multiplexed Magnetic Tweezers: From DNA Mechanics to Retroviral Integration*
	Jan Lipfert

Magnetic tweezers are a powerful tool to probe single DNA molecules and their processing by proteins under controlled forces and torques. Using a parallelized version of magnetic tweezers that can measure torque directly, we have carried out a series of high-precision torque and twist measurements of DNA mechanics and dynamics, which we use to critical test current simulations approaches for nucleic acids. Our results show that DNA twist changes with temperature[1] and ionic strength, while, in contrast, the intrinsic torsional stiffness is approximately independent of salt[2] and temperature. Quantitative comparison of high-resolution magnetic tweezers measurements to coarse-grained simulations of DNA mechanics shows that taking into account the anisotropy of DNA significantly improves agreement with torque measurements[3] and suggests an explanation of the large observed difference in buckling dynamics between DNA and RNA[4, 5].

Going beyond bare DNA, we have developed a magnetic tweezers assay to follow retroviral integration in real time, revealing the critical steps along the integration free energy landscape. In particular, we find an ultra stable strand transfer complex that suggests the role of a resolving factor *in vivo*.

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S 13	*Monitoring target search of a native CRISPR-Cas system in vivo*
	Johannes Hohlbein

Jochem Vink (TU Delft); Koen Martens (Wageningen University); Marnix Vlot (Wageningen University); Stan Brouns (TU Delft); JOHANNES HOHLBEIN (Wageningen University)

CRISPR-Cas systems in prokaryotes encode RNA-guided machineries that are tasked to find and cleave hostile invading DNA sequences shortly after cell entry. Here we investigated the target search process of native crRNA-effector complex Cascade inside cells and found that Cascade complexes survey cellular DNA by spending half their time rapidly probing DNA sequences at ~30 ms and the other half by freely diffusing in the cytoplasm. We found that CRISPR arrays themselves act as target decoys, and that target DNA transcription leads to Cascade release from bound targets leading to a binding affinity of 100-200 nM. We establish a relationship between Cascade copy number and CRISPR interference, and provide a novel mathematical framework describing the challenge of CRISPR target search in the crowded, DNA-packed environment of the cell.

S 14	*Folding dynamics of G-quadruplex DNA in dilute and molecularly crowded environments*
	Mikayel Aznauryan

G-quadruplexes are secondary nucleic acid structures that fold in the presence of physiological ions (Na^+ and K^+) on the basis of guanine-rich nucleic acid sequences. It is nowadays emerging that they play important regulatory roles in cell biology, are potential targets for anti-cancer therapies and can also be utilized as biocompatible structures in DNA nanotechnology.

Here we employed single-molecule Förster Resonence Energy Transfer (FRET) microscopy to investigate the folding and underlying conformational dynamics of human telomeric G-quadruplex DNA. Our results yield a comprehensive thermodynamic and kinetic description of the folding of G-quadruplexes that we find to proceed through a complex multi-route pathway, involving several conformational states. Our recent experiments in the presence of synthetic crowders probed the effect of macromolecular crowding on the folding of G-quadruplexes. Moreover, we compared our experimental data to the simplest model for the excluded volume effect on biomolecular processes - the scaled particle theory, and find a remarkable agreement between them. Altogether, these studies give a detailed picture of the folding dynamics of G-quadruplexes under broad experimental conditions thus uncovering common mechanistic features of their folding under cell-mimicking milieu.

I 17	*Exploring cell biology on a molecular level: Live-cell and quantitative localization microscopy*
	Ulrike Endesfelder

Ulrike Endesfelder, Max Planck Institute for Terrestrial Microbiology, Marburg

Our group develops and applies Single-Molecule Localization Microscopy (SMLM) techniques in cell biology - interested in the *in situ* observation of molecular processes in living cells.

SMLM data is built on single-molecule localizations, and thus allows determining the stoichiometry and molecular architecture of subcellular structures. Here, not only individual proteins can be precisely localized, but the large molecular architecture of multiprotein complexes or the organization of the genome can be targeted in the native cellular environment. This yields detailed quantitative molecular maps that capture these assemblies. In our vision, SMLM imaging thus has the potential to place hundreds of different molecules into assembled three-dimensional structures while maintaining the high spatiotemporal resolution of the present methods, ideally in correlative approaches.

Uniquely, SMLM can be combined with single-particle tracking (SPT) to measure a large batch of statistics on single-molecule dynamics inside living cells. It is thus possible to obtain spatially and temporally highly resolved diffusion maps that combine the multitude of single-molecule trajectories and accordingly unravel possible dynamic heterogeneities and subpopulations.

Behind todays attractive super-resolved images and analyses hides a rather high complexity of in large detail tailored experimental designs for specific organisms and environments. We cannot answer our research questions about the *in situ* behavior of molecular processes at a single molecules' spatiotemporal resolution without highly optimized and robust tools - which are still largely missing for most biological research fields.

In this talk, I will introduce some of our recently developed experimental and analytical tools alongside with our specific biological questions.

I 18	*Protein mechanics probed by high-speed force spectroscopy*
	Felix Rico

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The mechanical properties of individual proteins play a crucial role during essential mechanotransduction cell processes, such as migration, muscle contraction and adhesion. Thus, knowing the mechanical response at the single molecule level is important to understand biological function [1]. Atomic force microscopy (AFM) is a unique technology that combines nanometric-imaging capabilities with piconewton force resolution. However, AFM force measurements are often limited to millisecond time scales, while many biological processes occur at faster rates. We have recently adapted high-speed AFM for force spectroscopy to probe protein and cell mechanics at high rates with microsecond time resolution [2–4]. We applied high-speed force spectroscopy (HS-FS) to probe protein mechanics, including single protein unfolding and receptor/ligand unbinding, at the speeds of molecular dynamics simulations. This combined approach provides an atomic description of unfolding and unbinding processes based on experimental results. We propose HS-FS as a novel tool to confirm molecular dynamics simulations and to access new biophysical regimes relevant to biological function.

I 19	*Single Protein Dynamics:* *From Fluorescence To Electrical Detection*
	Sonja Schmid

Cees Dekker Lab, Bionanoscience Dept., Kavli Institute of Nanoscience Delft, TU Delft, NL
previous address: *Thorsten Hugel Lab, Phys.-Chem. Institute, Univ. Freiburg, D*

Proteins are the molecular makers in our body. They use diverse energy sources to perform specific tasks in a highly controlled and efficient manner. Biochemists have done a great job in identifying a vast proteome, with thousands of 3D structures, and a dense web of metabolic interactions characterized today. But for an in-depth understanding of the energetics and diverse driving forces that govern protein machines, we still lack detailed, dynamic information on the (sub-)molecular level.

Single molecule FRET [1] is amongst the most popular biophysical techniques to follow individual proteins at work in real time. Yet, due to photo-bleaching, the observation time of *one single molecule* spans hardly more than 2 orders of magnitude, e.g. 10ms - 1s, or 1s - 100s. This makes quantitative kinetic analysis challenging, and global interpretations based on one such 'time slice' are prone to artifacts. I will present here our recent solution to the challenge: a 2D machine-learning approach that extracts a maximum of information out of inherently noisy single-molecule trajectories [2]. It allowed us to pinpoint remarkable, mechanistic effects of the Hsp90 chaperone system [2-5].

Despite all experimental and analytical efforts, the temporal bandwidth of single-molecule fluorescence techniques remains poor, compared to protein dynamics occurring on diverse timescales simultaneously. A natural choice to study such broad-range dynamics is electrical detection, spanning >7 orders of magnitude (microseconds to hours) in one experiment. In an entirely new approach to protein kinetics, we employ a DNA-origami anchor to localize the protein of interest inside a nanopore. We monitor its behavior by means of conductance changes over time. I will present the current state of our label-free experiments on a transcription factor controlling anti-biotic resistance in bacteria. In combination with existing 3D structures and MD simulations, this new way of protein dynamics detection has the potential to provide the missing link to understanding the energetic origin of protein function at the sub-molecular level.

S 15	*Graphene – MIET: Optically Measuring Distances with Ångström Resolution*
	Arindam Ghosh

Arindam Ghosh, Akshita Sharma, Sebastian Isbaner, Ingo Gregor, Alexey I. Chizhik, Narain Karedla, Jörg Enderlein

Single-molecule fluorescence imaging has become an indispensable tool for almost all fields of research, from fundamental physics to the life sciences. Among its most important applications is single-molecule localization super-resolution microscopy (SMLM) (PALM [1] , STORM [2] , fPALM [3] , dSTORM [4] , PAINT [5]) which uses the fact that the center position of a single molecule's image can be determined with much higher accuracy than the size of that image itself. However , a big challenge of SMLM is to achieve super-resolution also along the third dimension. Recently, Metal-Induced Energy Transfer or MIET [6 , 7] was introduced as a technique to axially localize fluorescent emitters [8 , 9]. It exploits the energy transfer from an excited fluorophore to plasmons in a thin metal film. Here , we show that using graphene as the “metal” layer , one can increase the localization accuracy of MIET by nearly tenfold , and we demonstrate this by axially localizing single emitters and by measuring lipid bilayer thickness values with Ångström accuracy

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I 20	*Investigating synaptic adhesion dynamics and nano-organization using small monomeric binders*
	Ingrid Chamma

Ingrid Chamma, IINS, Bordeaux

Neuronal junctions are highly confined sub-micron scale structures containing thousands of proteins packed into a ~ 20 x 200-500 nm space. These proteins have to interact and organize into stable, yet plastic macromolecular platforms to mediate proper neuronal transmission, but remain rapidly adaptable to sustain developmental and activity-depend changes in the brain throughout life. The high protein density and the extreme confinement of such cellular compartments, make the internal organization of synaptic proteins difficult to decipher using classical fluorescence microscopy and conventional labeling tools. In this context, we have developed a new labeling method based on small monomeric binders that enable the investigation of synaptic protein organization at the nanoscale inside live synapses and live brain tissue. Using a combination of super-resolution microscopy techniques and monomeric binders, we showed efficient and specific targeting of adhesion proteins with high labeling density, reduced steric hindrance, and no cross-linking bias compared to large multivalent probes such as antibodies. This method allowed us to decipher for the first time the dynamic organization of major synaptic adhesion complexes.

I 21	*Spatial organisation and dynamics of GPCR signalling revealed by single-molecule fluorescence microscopy*
	Marie-Lise Jobin

Marie-Lise Jobin[1], Titiwat Sungkaworn[1], Donatella Treppiedi[2], Davide Calebiro[3]

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G protein coupled receptors (GPCRs) signalling cascades have been suggested to be organised in nanodomains at the plasma membrane, involving receptors, heterotrimeric G proteins and effectors but direct observation of such nanodomains has proven to be a major challenge. Our group has pioneered the use of innovative single-molecule microscopy methods, which allow us to investigate individual receptors and effectors on the surface of living cells with unprecedented spatiotemporal resolution (1).
Using this approach, we revealed both negative (2) and positive (3) interactions of receptors with the cytoskeleton, which control their subcellular localisation and influence their signalling. Moreover, we have previously shown that the γ-aminobutyric acid type B receptor (GABABR) dynamically interacts with the actin cytoskeleton (1), which might be important for its function. Therefore, we further investigated the underlying mechanisms using a combination of functional assays and single-molecule fluorescence microscopy. Site-directed mutagenesis on GABABR allowed us to identify the binding site on the receptor as well as the scaffold protein that mediates the cytoskeletal interaction. Additionally, our new results clarify the relevance of these interactions for the spatial localisation and downstream signalling of GABABR. These findings will help to better understand the dynamic spatiotemporal organisation of GABABR on the plasma membrane, which is likely to play an important role in assuring efficient coupling to downstream effectors and, thus, enable rapid and efficient synaptic modulation.

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2. Sungkaworn T, et al. (2017) Single-molecule imaging reveals receptor-G protein interactions at cell surface hot spots. Nature 550(7677):543-547.
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S 16	*Structural dynamics of single metabotropic glutamate receptors*
	Robert B. Quast

Robert B. Quast, Anne-Marinette Hanh Cao, Fataneh Fatemi, Philippe Rondard, Jean-Philippe Pin, Emmanuel Margeat

With some 800 members, G protein coupled receptors (GPCRs) constitute the largest membrane protein superfamily encoded in the human genome. The intrinsically allosteric activation mechanisms, by which an external signal is propagated across the membrane through conformational rearrangements, have been extensively studied over the last decades by sophisticated biophysical methods. These have led to the conclusion that GPCR activation cannot be sufficiently explained by a simple on/off transition from a static inactive to a distinct active state but is rather a highly dynamic process where the equilibrium between multiple coexisting conformational states is altered through interaction with ligands, lipids, and protein partners.
Among the various types of GPCRs, class C metabotropic glutamate receptors (mGluRs) are broadly distributed throughout the central nervous system and play an important role in the regulation of neurotransmitter release, neuronal excitability as well as cell depolarization. They have served in pioneering studies on GPCR structural dynamics by single molecule FRET, which until now have exclusively focused on the reorientation of the dimeric extracellular venus flytrap (VFT) domains, via stochastic N-terminal labeling.
In an effort to expand these studies and improve the understanding of structural dynamics for mGluR activation and signal transduction (and potentially GPCRs in general) we first evaluate different detergents with regard to the functional integrity of solubilized receptors by monitoring homogenous time-resolved ensemble FRET (HTRF) of N-terminally labeled receptor dimers in response to agonist-binding as well as positive and negative allosteric modulators.
Secondly, we use genetic code expansion, mediated by coexpression of orthogonal tRNA/synthetase pairs, to incorporate non-canonical amino acids (ncAAs) at selected positions within the receptors that enable site-specific attachment fluorophores at desired positions. Using smFRET combined with mulitparameter fluorescence detection (MFD), these tools will allow us to elucidate the correlated dynamics of the various structural domains of mGluR2 during its activation.

S 17	*Mechano-sensitivity of the bacterial flagellar motor*
	Francesco Pedaci

Ashley L Nord, Emilie Gachon, Ruben Perez-Carrasco, Jasmine A. Nirody, Alessandro Barducci, Richard M. Berry, and Francesco Pedaci

The bacterial flagellar motor (BFM) is the rotary motor that rotates each bacterial flagellum, powering the swimming and swarming of many motile bacteria. The torque is provided by stator units, ion motive force-powered ion channels known to assemble and disassemble dynamically in the BFM. This turnover is mechano-sensitive, with the number of engaged units dependent on the viscous load experienced by the motor through the flagellum. However, the molecular mechanism driving BFM mechano-sensitivity is unknown. Here, we directly measure the kinetics of arrival and departure of the stator units in individual motors via analysis of high-resolution recordings of motor speed, while dynamically varying the load on the motor via external magnetic torque. The data obtained indicate that the lifetime of an assembled stator unit increases when a higher force is applied to its anchoring point in the cell wall. This suggests that a catch bond (a bond strengthened instead of weakened by force) drives mechano-sensitivity of the flagellar motor complex. These results add the BFM to a short, but growing, list of systems demonstrating catch bonds, suggesting that this “molecular strategy” is a widespread mechanism to sense and respond to mechanical stress.

P 1	*Membrane-associated protein Mistic senses partial changes in lateral pressure profile of lipid bilayers*
	Marta Batet Palau

Marta Batet, Georg Krainer, Abhinaya Anandamurugan, Sandro Keller, Michael Schlierf

Mistic, a biofilm-promoting protein from *Bacillus subtilis*, is a unique protein among membrane-interacting proteins: It displays a high number of negatively charged residues on its surface, but surprisingly avidly associates with lipid-bilayer membranes. From previous studies in detergent micelles, it is known that Mistic's conformational stability is promoted by both polar interactions with detergent headgroups and hydrophobic contacts with moieties of the micellar core. However, little is known about Mistic's structural stabilization in lipid-bilayer membranes. In the present work, single-molecule FRET was used to monitor Mistic's conformational behavior in lipid-bilayer membranes. By reconstituting the protein into phospholipid vesicles of varying composition, it is shown that Mistic assumes two conformational states when interacting with lipid bilayers. Strikingly, by systematically varying acyl chain length, the degree of saturation, and membrane composition using binary lipid mixtures, the results suggest that the lateral pressure profile of the bilayer determines Mistic's conformational behavior and its switch between the two states. In the presence of bilayers with a higher lateral pressure on the acyl chains, Mistic is more likely to be found in an open, extended conformation, whereas in bilayers with a high lateral pressure on the headgroups Mistic adopts a more compact state. A model is proposed in which expanded Mistic resides within the interfacial region of the membrane, while the more compact conformation inserts deeper into the hydrophobic core of the bilayer.

P 2	*Comparison of GdmCl- and SDS-induced protein denaturation reveals massive acceleration of (un)folding kinetics in SDS*
	Vadim Bogatyr

V. Bogatyr, G. Krainer, A. Hartmann, J. Nielsen, D.E. Otzen, M. Schlierf

How does (un)folding of proteins in chemical denaturants compare with (un)folding in detergents? Here, we addressed this question by scrutinizing at single molecule level the equilibrium (un)folding dynamics of the ribosomal protein S6 in the presence of the chemical denaturant guanidinium chloride (GdmCl) and the surfactant sodium dodecyl sulfate (SDS). We subjected a fluorescently labeled protein variant to increasing concentrations of GdmCl or SDS and made use of single-molecule Förster resonance energy transfer (FRET) spectroscopy to probe its folding kinetics upon denaturation. We found that the protein (un)folds according to a two-state mechanism under both conditions, however, with greatly different kinetic rates: While S6 exhibits rather slow interconversion dynamics (e.g., slower than tens of milliseconds) in the presence of GdmCl, in accordance with previously obtained relaxation rates of 0.032 s^{-1} at midpoint conditions (i.e., 3.2 M GdmCl), the protein undergoes a massive speedup upon addition of SDS, with rate constants ms^{-1} (= 1000 s^{-1}) at 200 mM SDS), showing that the protein exhibits approximately 30'000-fold higher (un)folding rates in SDS compared with GdmCl. Nanosecond fluorescence correlation (nsFCS) experiments revealed that the speedup induced by SDS is paralleled by a marked speedup of polypeptide chain dynamics in the unfolded state compared with unfolded chain dynamics in GdmCl. Since chain reconfiguration times are correlated with the attempt frequency of barrier crossing, these findings may shed light on a possible mechanism for the tremendous increase of folding speed induced by SDS.

P 3	*Biophysical characterization of Vitamin D receptor activities modulated by RXR ligands*
	Sandra Chalhoub

S. Chalhoub, D. Rovito, G.Laverny, N. Rochel

The active form of vitamin D, calcitriol, is a key hormone for the regulation of calcium homeostasis. Calcitriol controls many biological functions, including the proliferation and differentiation of many cells, and has anti-inflammatory and immune activities modulatory. It regulates the expression of many target genes by binding to the nuclear receptor Vitamin D (VDR). VDR is thus an important therapeutic target for various pathologies (osteoporosis, neurodegenerative diseases, autoimmune diseases and cancers).

VDR acts as a heterodimer with the retinoid X nuclear receptors (RXRs), to control the transcription of target genes. Contradictory data in the literature about the role of RXR ligands Transcriptional Activity mediated by VDR. To study the effects of RXR ligands on the VDR interactions and transcriptional activities induced by various VDR ligands and to determine if RXR ligands can synergize ligand activities of VDR, we combined biophysical methods. Biacore Surface Plasmon Resonance and Micro Scale Thermophoresis have been used to quantify the effect of RXR ligand on DNAs binding and hetero-dimerization. For the DNA binding we choose different genes response elements characterized to be regulated by VDR.

P 4	*Free and Chaperone Bound Unfolded States of Outer Membrane Proteins*
	Neharika Chamachi

Neharika Chamachi, Georg Krainer, Pablo Gracia, Erik Frotscher, Andreas Hartmann, Philip Gröger, Sandro Keller, and Michael Schlierf

Structural and dynamic investigations of unfolded proteins are important for understanding protein-folding mechanisms as well as the interactions of unfolded polypeptide chains with other cell components. In the case of outer-membrane proteins (OMPs), unfolded-state properties are of physiological relevance, because they remain unfolded for extended periods of time during their biogenesis and rely on interactions with periplasmic chaperones to prevent aggregation and support correct folding. Here, we study the unfolded-state properties of a 12 beta-stranded outer-membrane phospholipase A (OmpLA) both in its free and chaperone-bound states. Using a combination of ensemble and single-molecule spectroscopy techniques including single-molecule FRET, we find that under strongly denaturing conditions and in the absence of chaperones, OmpLA populates an ensemble of slowly (>100 ms) interconverting and conformationally heterogeneous unfolded states that lack the fast chain reconfiguration motions expected for an unstructured, fully unfolded chain. Interestingly, when complexed with periplasmic chaperones seventeen kilodalton protein (Skp) and survival factor A (SurA), OmpLA adopts an expanded, yet squished conformational ensemble, in which the overall broad distribution of unfolded states is preserved. Differently sized OMPs like OmpX (8 beta-strands) and OmpF (16 beta-strands) were used to gather a broader perspective on chaperone enabled conformations of OMPs. These findings could indicate that periplasmic chaperones Skp and SurA prestructure OMPs for their next binding partner (e.g., the β-barrel assembly machinery (BAM)) to facilitate insertion and proper folding into the outer membrane.

P 5	*Influenza A matrix protein (M1) multimerization is the main force driving membrane bending and tubulation*
	Ismail Dahmani

The matrix protein of the Influenza A virus (M1) forms a shell underlying the viral lipid envelope and controls the geometry of the virus capsid. In infected cells, M1 orchestrates the process of new virion formation by binding to the inner leaflet of the plasma membrane (PM), finally inducing bending of the lipid bilayer and virus release. The exact role of M1 polymerization in inducing membrane deformation and budding is not clear. As a first step to model virus egress through the PM, we analyzed M1 binding to giant unilamellar vesicles (GUVs). Our results show that M1 binds to negatively charged lipids, causing deformation by imposing curvature and membrane tubulation, even at high cholesterol contents. Detergent-mediated solubilization of the lipid bilayer of the deformed vesicles after M1wt (or N-terminal domain) binding leaves the three-dimensional organization of the protein layer intact like a capsid, indicating that M1 forms a very stable 2D network adjacent to (but independent from) the lipid membrane by *multimerization*. This tendency for M1 to induce tabulation was also confirmed by Cryo-TEM with liposomes. Secondly Experiments of M1 binding to vesicles under conditions inhibiting M1 *multimerization* like at acidic pH (pH=5) or in presence of protein interaction inhibitor drug (PHE) showed that M1 irreversibly loses its ability to induce curvature. We also performed Surface Plasmon Resonance (SPR) analysis to measure the membrane binding of M1 [± drug 100 µM]. Single FCS measurement and FRAP analysis also confirmed further the correlation between the natural tendency of the protein M1 for oligomerization and membrane deformation.

P 6	*Illuminating Auxin Response Factor binding equilibrium and kinetics at the single-molecule level*
	Mattia Fontana

Mattia Fontana, Willy van den Berg, Dolf Weijers, Johannes Hohlbein

Auxin signalling plays a role in regulating almost every aspect of plant growth and development. The transcriptional response to auxin is mediated mainly via proteins belonging to the Auxin Response Factor (ARF) family.

Structure-based model of ARF's DNA-binding-domain (DBD) shows that regulation of target genes requires both protein-DNA interaction as well as protein dimerization; nevertheless information about the dynamics of these interactions is still missing.

We developed fluorescence-based assays to quantify the dynamics of these inter-molecular interactions at the single-molecule level. We used fluorescently labelled dsDNA (~40bp) containing variations of the auxin response element (AuxRE); we then applied techniques such as single-molecule Förster resonance energy transfer (smFRET) alone and in combination with protein induced fluorescence enhancement (smPIFE-FRET) to characterize the binding kinetics of ARF-DNA complexes. We plan to study the binding equilibrium and kinetics of various ARF-RE pairs; this will provide novel insights into the binding behavior of this important transcription factor.

P 7 / T1	*Angstrom precision distance measurements in dynamic protein structures with FRET*
	Christian Gebhardt

Christian Gebhardt, Rebecca Mächtel, Niels Zijlstra, Marijn de Boer, & Thorben Cordes

Single-molecule Förster resonance energy transfer (smFRET) has evolved towards a mature toolkit for the study of distances, structures and dynamics of biomolecules in a physiologically relevant context in vitro and in vivo. There is, however, no generally accepted way to derive and use quantitative distance information from the FRET-ruler to derive structural models or constraints in the protein data base. Hellenkamp et al. recently presented a quantitative smFRET study of artificial DNA-ruler structures that revealed high precision, accuracy and reproducibility of FRET-derived distances in a worldwide comparative study of 20 labs with a distance uncertainty of less than 6 Å[1]. While this establishes smFRET as a suitable technique for accurate distance measurements of static biological reference structures, we raise the question if smFRET is applicable for proteins with dynamic conformational motions or allosteric modulation of protein structure by an effector. Additionally, proteins are more challenging targets for site-specific fluorophore labelling compared to oligonucleotides. We identified a suitable model system that we used here to benchmark FRET-derived distance uncertainty in proteins for situations of (i) stochastic labelling and (ii) allosteric and dynamic modulation of the structure and show similar angstrom precision within more than 15 labs comparable to DNA[2].

1. Hellenkamp, B. *et al.* Precision and accuracy of single-molecule FRET measurements—a multi-laboratory benchmark study. *Nat. Methods* **15,** 669–676 (2018).
2. Gebhardt, C. *et al. How accurate is single-molecule Förster-resonance energy transfer for dynamic protein structures?* (2019, in preparation).

P 8	*Studying chromosome organization using multiplexed 3D super-resolution microscopy*
	Markus Götz

Markus Götz, Emmanuel Margeat, Marcelo Nollmann

The genomic information of eukaryotic cells is stored in a highly non-random fashion inside the nucleus. Multiple levels of chromatin organization, from nucleosomes to chromosome territories, exist and are involved in key cellular functions, such as transcriptional regulation, cell fate decision or DNA replication. On the kilo- to megabase pair scale, chromatin forms topologically associating domains (TADs), regions that are self-interacting and span the size of individual genes or regulatory domains. TADs also coincide with functional epigenetic domains defined by chromatin marks.

The recently developed, multiplexed and sequential imaging approach (Hi-M) permits the simultaneous detection of chromosome organization and transcription on the single cell level (1). The genomic DNA is fluorescently labeled in a consecutive manner with oligonucleotide-based probes (“oligopaint”). This allows visualizing the trajectory of the chromatin fiber in the nucleus with kilobase pair resolution. As the method is microscopy-based, spatial information is maintained and cell-to-cell variations can be investigated with respect to the whole organism under study. Currently, we are expanding this approach by using super-resolution methods. This will permit us to gain an even more detailed view on the structural organization and dynamics of chromatin and its implications on DNA processes during the cell cycle.

1.Gizzi, A.M.C., D.I. Cattoni, J.-B. Fiche, S. Espinola, J. Gurgo, O. Messina, C. Houbron, Y. Ogiyama, G.-L. Papadopoulos, G. Cavalli, M. Lagha, and M. Nollmann. 2018. Microscopy-based chromosome conformation capture enables simultaneous visualization of genome organization and transcription in intact organisms. bioRxiv. : 434266.

P 9	*Regulation of the Blood Protein von Willebrand Factor under Low Forces*
	Sophia Gruber

Sophia Gruber,[1] Achim Löf,[2] Tobias Obser,[2] Reinhard Schneppenheim,[2] Maria A. Brehm,[1] Martin Benoit,[1] Jan Lipfert[1]

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Von Willebrand factor (VWF) is a large, multimeric vascular glycoprotein that is crucially involved in primary and secondary haemostasis. Upon sensing increased hydrodynamic forces – as present at sites of vascular injury – VWF undergoes multiple conformational changes that expose binding sites for platelets and collagen and thus activate VWF for its haemostatic function. The smallest repeating subunits of the long linear multimers are dimers formed by two C-terminally linked multi-domain monomers. AFM imaging suggests that one of the first regulatory responses of VWF to low force is unzipping of the dimeric “stem”, formed by the C-terminal domains of both monomers. Direct investigation of this unzipping, however, has been difficult as relevant forces are in the range of 1 pN and thus below the force range accessible by most methods of single-molecule force spectroscopy. We established a magnetic tweezers assay that allows highly parallel measurements on a large number of single proteins under constant forces with excellent resolution at low forces (0.1 pN).We employed this approach to investigate stem unzipping and re-zipping in dimeric VWF. In a physiologically relevant force range between approximately 0.5 and 1.5 pN, we observed fast and reversible transitions between multiple states that we identify with stepwise unzipping and re-zipping of stem domains. In order to reliably assign the transitions and to dissect the contribution of different domains, we are currently characterising the zipping under different ionic conditions and pH values, investigating the previously described pH-dependency of stem formation. Additionally, we are aiming at characterising zipping in VWF variants with domain deletions and with clinically relevant mutations located in the stem region. We are further improving the transition analysis by implementing a hidden Markov model approach to precisely analyse the number of states as well as improve kinematic modelling.

P 10	*Observing Biomolecular Dynamics from Nanoseconds to Hours with Single-Molecule Fluorescence Spectroscopy*
	Andreas Hartmann

Andreas Hartmann, Georg Krainer, Simon Ollmann, Pablo Gracia, Michael Schlierf

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The intricate process of molecular self-assembly, also termed folding, is crucial for the proper functioning of biomacromolecules. Acquired gene variations as well as abnormal amino acid modifications in proteins and other biomolecules can cause misfolding resulting in a myriad of pathological conditions. Thus, a better understanding of the thermodynamic and kinetic fundamentals of inter- and intramolecular conformational changes are necessary to shed light on underlying disease mechanisms. During the past decades, numerous fluorescence techniques evolved in the field of molecular biology paving the way for observations of conformational changes at various timescales. However, a unified approach covering most biologically relevant timescales remains a missing link in the field of fluorescence spectroscopy. This would comprise a methodological workflow for the qualitative and quantitative analysis of biomolecular dynamics ranging from nanoseconds to hours.

In this work, an integrated approach for single-molecule fluorescence spectroscopy studies is presented that enables the observation of folding dynamics spanning more than nine orders of magnitude. To this end, a custom-built single-molecule fluorescence spectroscopy setup was constructed combining single-molecule FRET spectroscopy, time-correlated single photon counting, fluorescence correlation spectroscopy, and fluorescence anisotropy techniques for multiparameter fluorescence detection. The setup allows the complementary observation of single molecules over an extensive timescale ranging from fast nanosecond-timescale polymer dynamics to slow hour-timescale protein-folding processes without the need of synchronization. Observations of freely diffusing molecules enable high throughput measurements in heterogeneous membrane-mimetic and denaturing environments. Additionally, routines for data acquisition and processing were developed followed by the elaboration of a methodological workflow for the qualitative and quantitative analysis of biomolecular dynamics and kinetic rate constants. Capitalizing on these developments, the broad applicability of the methodology was applied to a diverse set of biomolecular processes including DNA hairpin folding, Holliday junction conformational changes, as well as soluble and membrane-protein folding dynamics in various environments.

P 11	*Elucidating Zinc Binding to the Voltage-Gated Proton Channel hHv1 Using Computersimulations*
	Christophe Jardin

Christophe Jardin, Boris Musset

H_V1 voltage-gated proton channels are proton-specific ion channels with unique properties. For example, they are massively expressed in human sperm where they are necessary for maturation and motility, hence essential for conception.

Voltage-gated proton channels are strongly inhibited by Zn^{2+}. Experimental studies revealed that histidine residues are essential for Zn^{2+} binding. However, the two accessible histidine residues H140 and H193 are too far apart to coordinate simultaneously one Zn^{2+} in a structural model of the monomeric channel. It was thus hypothesized that two Zn^{2+} binding sites can be formed between pairs of equivalent histidine residues (H140-H140, and H193-H193) at the interface of a H_V1 homodimer. The consecutive experimental measurements were also in agreement with this hypothesis.

We tested this hypothesis and investigated the determinants of Zn^{2+} binding at the molecular level using computational approaches: molecular modeling, molecular docking, and molecular dynamics simulations.

Our results support the hypothesis enunciated above: The modeling and docking simulations show that the hH_V1 channels can form homodimers that present an appropriate interface for two Zn^{2+} binding sites, each involving a pair of equivalent histidine residues from each monomer. The molecular dynamics simulations reveal that two Zn^{2+} can stably be accommodated in the proposed binding sites. The zinc ions are coordinated by the histidine and two acidic residues. Essentially, the glutamate residues E192 play an essential role in Zn^{2+} binding. Comparison with another possible dimer conformation and with the monomeric form of the channel also reveals why the dimer conformation hypothesized above is more able to coordinate zinc ions.

P 12	*Structural investigations of highly pathogenic negative strand RNA viruses*
	Janine-Denise Kopicki

Janine-Denise Kopicki[1], Johannes Heidemann[1], Tobias Holm[2], Stephan Günther[2], César Muñoz-Fontela[2], Sophia Reindl[2], Maria Rosenthal[2], Dominik Vogel[2], Charlotte Uetrecht[1,3]

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Facing the Ebola crisis in 2014, the WHO encouraged research on pathogens with high epidemic potential, especially as vaccines and effective treatments are often not yet available. Some highly pathogenic representatives of the negative strand RNA viruses, such as Ebola and Marburg virus (EBOV; *Filoviridae*), Lassa virus (LASV; *Arenaviridae*) or Crimean Congo haemorrhagic fever virus (CCHFV; *Bunyaviridae*) entail an increased risk. Structural elucidation of viral assembly as well as transcription and replication mechanisms are fundamental for developing anti-viral strategies and ultimately taking countermeasures with drugs to protect society and economy in the affected regions. Native mass spectrometry (MS) gives us dynamic insights into the molecular interactions and mechanisms of the viral life cycle.

It is of particular interest for us to elucidate the assembly mechanism of the EBOV nucleocapsid. Another focus is the investigation of interactions within the replication complexes of Arena- and Bunyaviruses. In this context, we have already investigated some essential proteins of the replication machinery of LASV, namely its polymerase, the nucleoprotein and the Z-protein. So far, we have also been able to contribute to the structural analysis of CCHFV cap-snatching endonuclease, which is a part of the viral polymerase using native MS.

P 14	*FRET-Based Study of the Dynamic Structure of a Two-Domain Protein*
	Alexander Larbig

Alexander Larbig, Bianca Reschke, Jakub Kubiak, Mykola Dimura, Daniel Mulnaes, Claus A. M. Seidel

In this work, FRET is used to investigate the dynamic structure of the two-domain protein CBM56, the carbohydrate-binding module of a beta-1,3-glucanase. The structural information is obtained by measuring distances between different FRET pairs using multiparameter fluorescence spectroscopy, which complement the computational structure prediction based on the amino acid sequence. The work includes FRET pair prediction using NMSim coarse-grained simulations to obtain the most meaningful dye positions, the method of gene expression and protein purification to produce the target protein, the performance of the FRET measurements and the analysis of the data for highly accurate structure modeling.

P 15 / T 2	*Coiled Coils As Structural Building Blocks: A Sequences-Based Approach Towards Tunning Coiled Coil Mechnics*
	Patricia Lopez-Garcia

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The natural abundance of coiled coil (CC) motifs in cytoskeleton and extracellular matrix proteins suggests that CCs play an important role as passive (structural) and active (regulatory) mechanical building blocks. It is well established that modifications in the CC sequence, e.g. in hydrophobic core or solvent-exposed residues, are responsible for the thermodynamic stability of CCs; however, nothing is known about how these factors affect CC mechanics.

With the goal of shedding light on the sequence-structure-mechanics relationship of CCs we have chosen thermodynamically well-characterized sequences and analyzed their mechanical stability using single molecule force spectroscopy, applying force parallel to the helical axis in the 'shear' geometry. Modifications in the hydrophobic core or the helix propensity both alter the binding potential, but with different outcomes: a less tightly packed hydrophobic core increases the potential width (Δx), without significantly affecting the barrier height (k_{off}). In contrast, a reduced helix propensity decreases both potential width and barrier height. Our goal is to use this information for developing a library of mechanically characterized CCs that can be applied as calibrated building blocks for a wide range of applications: from molecular force sensors to mechanosensitive material crosslinks in protein nanostructures and synthetic ECM mimics.

P 16	*Studying multimeric G-quadruplex formation using single-molecule FRET*
	Simon Ollmann

Simon Ollmann, Georg Krainer, Andreas Hartmann, Michael Schlierf

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Telomeres are genomic regions found at the ends of eukaryotic chromosomes. Their single-stranded overhangs are composed of arrays of guanine-rich repeats, which can fold into various structural conformations, including so-called G-quadruplexes (G4s). G4s are polymorphic secondary structure elements that are important regulatory factors for genome stability and promising drug targets for cancer therapy. In recent years, important molecular insights into the dynamics and structural polymorphisms of G4 have been obtained using single-molecule techniques. So far, most single-molecule studies have been limited to short sequences composed of four tandem TTAGGG repeats capable of forming single G4 units. Yet, human telomeres have protruding single-stranded overhangs of up to 200 nucleotides containing tens of copies of the sequence TTAGGG, which can fold into arrays of multimeric G4 structures. Here, we employed single-molecule Förster resonance energy transfer (FRET) microscopy to study the folding and dynamics of multimeric G4 structures. Specifically, we probed single-unit G4 conformations and their dynamics in the context of the G4-triad-forming $(TTAGGG)_{12}TT$ construct. Our results suggest that single G4 units within the G4 triad are destabilized compared to monomeric G4s and show a highly polymorphic structural behavior depending on salt condition and relative position within the G4 array. Folded conformations are observed in the presence of Na^+ as well as Li^+ cations. Our study provides first insights into the rich conformational diversity of multimeric G4 structures and their associated dynamics. Future studies will investigate the influence of telomeric proteins on multimeric G4 formation, dynamics, and topology.

P 17	*Extending the FRET range limit using a zero mode waveguide aperture*
	Satyajit Patra

Satyajit Patra, Mikhail Baibakov, Jean-Benoit Claude and Jerome Wenger

Single molecule Förster resonance energy transfer (FRET) is a powerful biophysical method to investigate the structural dynamics of a biomolecule. However, optical measurements of FRET are limited to a distance range below 10 nm and therefore can not probe long distance dynamics.

Here, we have used a single aluminum zero mode waveguide (ZMW) to extend the FRET distance range and enhance the FRET efficiency of a double stranded DNA with a 13.6 nm separation between donor (Atto 550) and acceptor (Atto 647N) on the single molecule level. A single step surface passivation using polyethylene glycol silane is found to efficiently eliminate the adhesion of the DNA inside the ZMW.

We have recorded a 10-fold enhancement in the single molecule fluorescence intensity together with a 3-fold decrease in fluorescence lifetime for both Atto 550 (donor) and Atto 647N (acceptor) labelled DNA in the presence of 120 nm and 140 nm diameter nanoholes. A 2-fold enhancement in the FRET efficiency from 2.5 % to 5 % is also observed for the DNA in the presence of 120 and 140 nm nanoholes at large D-A separations exceeding 13 nm. This study paves the way for pushing the limit of FRET towards longer distances at physiologically relevant conditions.

P 18	*Mobility of LexA during SOS response revealed by single-molecule microscopy in living bacteria*
	Leonard Schärfen

Leonard Schärfen, Milos Tisma, Michael Schlierf

Emergence of antibiotic resistance among pathogenic bacteria remains a major challenge in modern medicine. Recent efforts have focused on understanding and preventing the rapid evolution towards persistence against commonly used antibiotics. A key mechanism driving mutation and adaptation associated with antibiotic stress is the SOS response. The central mediator of this gene regulatory network is the transcriptional repressor LexA, which is auto-proteolytically cleaved upon DNA damage and consequently allows transcription of ~40 SOS operons, including error-prone polymerases and recombinases. Through fusion of the chromosomal *lexA* gene with photoactivatable fluorescent proteins in *Escherichia coli*, we follow the diffusion of individual LexA molecules using single-molecule tracking photoactivated localization microscopy (smtPALM) in living cells. By quantifying different modes of diffusion which the transcription factor undergoes, we give precise measures of SOS response intensity, drawing a more detailed picture of the events taking place when bacteria are exposed to DNA damage. We found that a great fraction of unbound LexA molecules stay associated with the nucleoid, possibly sampling chromosomal DNA for its binding site. We investigated the exact timing of SOS induction, and provide timescales for individual layers of regulation. Additionally, we acquire single-molecule localization images of LexA in fixed bacterial cells, in which we observe transcription factor clustering. Ultimately, using careful genetic and environmental perturbations, we aim to characterize the SOS response on a dynamic and quantitative molecular scale.

P 19	*Molecular insights into the structural effects of a pathogenic CFTR loop mutation and the action of a pharmacological corrector*
	Mathias Schenkel

Mathias Schenkel, Georg Krainer, Charles M. Deber, Michael Schlierf

To drive the rational design of cystic fibrosis (CF) therapies, it is important to elucidate how mutational defects in CFTR lead to its impairment, and how pharmacological compounds interact with CFTR. Here, we investigate the structural effects of the patient-derived CF-phenotypic mutation E217G located in the loop region of CFTR's membrane-spanning domain using a helical-hairpin construct derived from CFTR's transmembrane (TM) helices 3 and 4 (TM3/4) and their intervening loop. We employ a single-molecule FRET assay to probe the folding status of reconstituted hairpins in lipid bilayers. We find that the E217G hairpin exhibits an altered adaptive packing behavior stemming from an additional GXXXG helix–helix interaction motif created in the mutant hairpin. This suggests that the misfolding and functional defect caused by the E217G mutation arises from an impaired conformational adaptability of TM helical segments in CFTR. Addition of the small-molecule corrector Lumacaftor exerts a helix stabilization effect not only on the E217G mutant hairpin but also on WT TM3/4 and other mutations in the hairpin. This suggests that Lumacaftor has a general mode of action likely connected to its membrane-destabilizing properties, through which it efficiently improves maturation of various CFTR mutants.

P 20 / T 3	*Infrared study of the protein GlcPSe and determination of pKa*
	Ana F. S. Seiça

Ana F. S. Seiça, Cristina V. Iancu, Jun-yong Choe and Petra Hellwig

Staphylococcus epidermidis glucose/H^+ symporter ($GlcP_{Se}$) a membrane transport protein belonging to the major facilitator superfamily (MFS) is highly specific for glucose and homolog to the human glucose transporters. Aspartic acid residue (Asp22), an important residue of $GlcP_{Se}$ involved in proton (H^+) binding site, is located closer to the inner gate of the glucose binding site and the putative inner gate of the glucose transporter [1]. This residue is discussed to rule the proton translocation.

Surface-enhanced infrared absorption spectroscopy (SEIRA) of the immobilized $GlcP_{Se}$ on a modified gold layer [2] in an attenuated total reflectance (ATR) was used to study the pH- and substrate-dependent conformational changes of the protein.

The residue Asp22 exhibits a pKa of 8.2 ± 0.2, indifferent to the presence of glucose, similar to the protein Lactose Permease (LacY) where the pKa of the aminoacid Glu325 determined in situ by infrared spectroscopy was proven to be insensitive to galactopyranoside [3].

A neutral replacement of the negatively charged Asp22 lead to positive charge displacements over the entire pH range, suggesting that the polarity change of the *wild type (WT)* reflects the protonation state of Asp22.

Mutation of further residues involved in the H^+ binding site were studied. Ile105Ser had no effect on the pKa value of the carboxylic group at position 22. Ile105Ser has a phenotype similar to *WT,* binding sugar in the protonated and deprotonated form. The effect of further variants on proton translocation will be discussed.

[1] Iancu, C. V., Zamoon J., Woo, S. B., Aleshin, A., Choe, J.Y. (2013) Crystal structure of a glucose/H^+ symporter and its mechanism of action. PNAS, vol.110, no.44:17862-17867

[2] Ataka K., Heberle J. (2006) Use of surface enhanced infrared absoprtion spectroscopy (SEIRA) to probe the functionality of a protein monolayer. Biopolymers 82(4) 415-419

[3] Grytsyk, N., Sugihara, J., Kaback, H. R., Hellwig, P. (2016) pKa of Glu325 in LacY. PNAS vol.114, no.7:1530-1535

P 21	*Fast, high yield and simple design for single-molecule force spectroscopy nucleic acid scaffold fabrication.*
	Flavia Stal Papini

Flavia Stal Papini, Mona Seifert, David Dulin

Force spectroscopy single-molecule studies of nucleic acid processing enzymes, e.g. helicase, polymerase, require the development of complex nucleic acid scaffolds to load the enzyme and monitor its forward translocation. For example, DNA hairpins have been developed to study the unwinding kinetics of replicative helicases. For short nucleic acid hairpins, i.e. with a stem 50 bp, it is relatively straightforward to design, buy and hybridize oligonucleotides to form the desired hairpin; however, the procedure is far more complex when the hairpin design contains ~1 kbp stem: multiple hybridization-ligation-purification steps are then necessary to obtain a scaffold with high purity, though with low yield.

Here, we present several protocols to make DNA/RNA scaffolds, either double stranded coilable linear constructs, or hairpins with a stem of 0.5-1 kbp length. To demonstrate the effectiveness of our approaches, we performed force-extension and rotation-extension experiments using magnetic tweezers. In particular, we designed new protocols - based on commercially available reagents - to fabricate long stem DNA and RNA hairpins with higher yield and purity in fewer steps than already existing protocols.

We believe that these results will be extremely valuable to the community and broadly applicable to single-molecule force spectroscopy experiments.

P 22	*Correlative single-molecule localization and electron microscopy of biosilica forming proteins*
	Adeeba Fathima

Adeeba Fathima, Nicole Poulsen, Nils Kröger, Michael Schlierf

Diatoms are single celled algae which possess the intriguing ability to form nanoscale biosilica cell walls with intricate species-specific hierarchical structures. The biogenesis of these cell walls is presumably a self-assembly process by several biomolecules. We use correlative single-molecule localization fluorescence and transmission electron microscopy (sCLEM) for resolving protein nano-patterns in diatom biosilica cell walls. We dissolve the biosilica cell walls on a carbon-coated TEM grid and subsequently localize the distribution of a protein of the Cingulin-family in the organic matrix using Stochastic Optical Reconstruction Microscopy (STORM). After a complete localization, we obtain a corresponding transmission electron microscopy image which is aligned with fiducial markers. While electron microscopy allows nanometer resolution of the isolated protein matrix, it does not allow protein specific labeling. STORM allows sub-diffraction imaging of a specific fluorescently labelled protein, thereby allowing to localize proteins of interest within the protein matrix. Identifying the patterns formed by different proteins and their correspondence to the physical structure of the biosilica from the information rich sCLEM images can shed light on their roles during the self-assembly process and during the formation of different morphological features of the diatom biosilica.

P 23 / T 4	*Structural analysis of nuclear pore complex formation in nuclear envelope by AFM / Fluorescence correlative microscopy*
	Anthony Vial

Anthony Vial, Pierre-Emmanuel Milhiet, Christine Doucet

Nuclear pore complexes are the only gateways between the nucleus and cytoplasm in eukaryotic cells. Their integrity is essential to ensure protection of the genome and proper gene expression. As a matter of fact, alterations of the nuclear permeability barrier participate in the development of diseases such as cancer.

Very little is known about early events of interphase pore assembly, in contrast with post-mitotic pore assembly, which is rather well understood. This field is lagging behind, mostly due to a lack of tools to monitor the formation of early pore intermediates.

To study this pore assembly in interphase, we use Atomic Force Microscopy (AFM) coupled with fluorescence microscopy to measure the topography of native sites of formation on open cell nuclei.

With this method, we obtained preliminary results that show high resolution topography of the nuclear pore complexes visualized from the inner side of the nuclear membrane of open nuclei. We could image the nucleoplasmic side of the nuclear envelope and resolve details in the nuclear pore complex basket. In addition, we started to measure topography associated to native sites of formation thanks to correlation of the AFM images with fluorescence signal of labeled nuclear pore complexes. These results show that we can use this method to measure membrane topography of native sites of nuclear pore complex assembly to determine the kinetics of recruitment of different implied proteins.

P 24	*Microsecond-Scale Molecular Dynamics Simulations and Single Molecule FRET on the Allosteric Action of Nucleotides on the Heat Shock Protein 90 Dimer*
	Steffen Wolf

Steffen Wolf[1], Benedikt Sohmen[2], Björn Hellenkamp[2], Thorsten Hugel[2], Gerhard Stock[1]

[1] Biomolecular Dynamics, Institute of Physics, Albert-Ludwigs-University Freiburg
[2] Institute of Physical Chemistry, Albert-Ludwigs-University Freiburg

Ligand binding to proteins and subsequent functional control by structural changes is at the heart of regulation of protein function. Here, we report on a combination of extensive unbiased all-atom molecular dynamics simulations (25 μs total simulated time) and single molecule FRET experiments with the full 1300 amino acid Heat Shock Protein 90 (Hsp90) dimer. Hsp90 is one of the most conserved heat shock proteins, and besides its role as chaperone for a wide range of substrates serves as regulator for protein kinases. We show how external energy input in the form of ATP puts the Hsp90 dimer under strain, and forces the protein into an energetically disfavored active folding conformation. Interestingly, only few amino acids appear to be responsible to mediate this conformational shift between nucleotide binding pocket and the full protein dimer. Hydrolysis of ATP to ADP + $P_i$removes the strain from the protein, causing a relaxed, inactive conformation. The transition of structural information from initial nucleotide/amino acid contact loss at the nucleotide binding site to complex structure changes of the full dimer structure follows hierarchical dynamics.

C 4	*DYNAMIC BIOSENSORS - ANALYZING MOLECULES IN MOTION*
	Dynamic Biosensors

Dynamic Biosensors is a provider of instruments, consumables, and services in the field of analytical systems for the characterization of biomolecules and molecular interactions.

The company is located in Martinsried, south of Munich, a vital center of Europe's biotechnology industry. Dynamic Biosensors commercializes switchSENSE® technology, a groundbreaking platform technology for the analysis of biomolecules with applications in R&D and drug development.

The switchSENSE® technology is protected worldwide and only available through Dynamic Biosensors. The company is headquartered in the south of Munich, Germany and runs offices in the United States, the United Kingdom, Japan and Singapore.

C 5	*Investigating Dynamic Biological Processes with High-Speed, High-Resolution Correlative AFM-Light Microscopy*
	Sandra Kostrowski JPK BioAFM Center

The ability of atomic force microscopy (AFM) to obtain three-dimensional topography images of biological molecules and complexes with nanometer resolution and under near-physiological conditions remains unmatched by other imaging techniques. However, the typically longer image acquisition times required to obtain a single high-resolution image (~minutes) has limited the advancement of AFM for investigating dynamic biological processes. While recent years have shown significant progress in the development of high-speed AFM (HS-AFM), the ability to scan faster has typically been achieved at the cost of decreased scanner range and restricted sample size. As such, these HS-AFM systems have mainly focused on studying single molecule dynamics and have been very limited in their ability to conduct live cell imaging.

The novel NanoWizard® ULTRA Speed AFM not only enables high-speed studies of time-resolved dynamics associated with cellular processes, it's latest scanner technologies and compact design also allow full integration of AFM into advanced commercially available light microscopy techniques. Thus, fast AFM imaging of several frames per second can be seamlessly combined with methods such as epi-fluorescence, confocal, TIRF, STED microscopy, and many more. We will present how the latest advances in the ULTRA Speed AFM are being applied to study a wide-range of biological samples, from individual biomolecules to mammalian cells and tissues. We will also describe how this unique system is enabling new research opportunities with high-speed, high-resolution correlative AFM-light microscopy.

C 6	
	NIKON

Firmenprofil Nikon GmbH

Die Nikon GmbH in Düsseldorf (gegründet 1971) ist die Vertriebs- und Serviceniederlassung für Mikroskope, Fotokameras und Scanner in Deutschland.

Unsere Produktpalette Mikroskope umfasst ein breites Spektrum an modernen Lichtmikroskopen, Stereomikroskopen und Digitalkameras für Labor und Praxis.

Außerdem bieten wir Ihnen komplexe optische Systeme für die medizinische Forschung. Die Verbindung von robuster Mechanik, Spitzenoptik und moderner Elektronik für Steuerung und Bildanalyse führt zu leistungsfähigen und ergonomischen Mikroskopen. Das gilt für Anwendungen in der Biomedizin genauso wie für Anwendungen in der industriellen Qualitätssicherung und Materialforschung.

C 7	
	PicoQuant

PicoQuant is a Berlin (Germany) based, leading research and development company specializing in optoelectronics. The product portfolio encompasses picosecond pulsed diode lasers, photon counting instrumentation, fluorescence lifetime spectrometers, FLIM and FCS upgrade kits for laser scanning microscopes as well as time-resolved confocal and super-resolution microscopes. A variety of fluorescence spectrometers is offered that ranges from compact table-top systems for education or daily routine work to modular, fully automated high-end spectrometers with excellent sensitivity and time resolution. The available microscopy systems include complete single molecule sensitive confocal or super-resolution microscopes with picosecond temporal resolution as well as FLIM and FCS upgrade kits for laser scanning microscopes from the major manufacturers.